Adenoviruses – Infection, pathogenesis and therapy

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Abstract: Both well-known and emerging viruses increasingly affect humans and cause disease, sometimes with devastating impact on society. The viruses present in the biosphere are the top predators in the life chain, virtually without enemies, except perhaps the immune system, and harsh environmental physicochemical conditions restricting their dissemination. We know a lot about viruses, but do we know enough? This series of reviews is dedicated to adenoviruses (AdVs), a family of nonenveloped DNA viruses occurring in vertebrates, including humans. AdVs have been the focus of intense research for more than 67 years. Besides causing disease, they have immensely contributed to the advance of life sciences and medicine over the past decades. Recently, AdVs have been widely used as vehicles in gene therapy and vaccination. They continue to provide fundamental insights into virus-host interactions in cells, tissues and organisms, as well as systems and metabolic networks. This special issue of FEBS Letters presents a unique collection of 23 state-of-the-art review articles by leading adenovirologists. In this prelude, I present the chapters, which provide a solid basis for further exploring the rich heritage in adenovirus molecular cell biology, structural biology, genetics, immunology, gene therapy and epidemiology. I conclude with an essential discussion of six blind spots in adenovirology.

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EDITORIAL

Adenoviruses - Infection, Pathogenesis and Therapy

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Abstract

Well-known and emerging viruses increasingly affect humans and cause disease, sometimes with devastating impact on society. The viruses present in the biosphere are the top predators in the life chain, virtually without enemies, except perhaps the immune system, and harsh environmental physico-chemical conditions restricting their dissemination. We know a lot about viruses, but do we know enough? This series of reviews is dedicated to adenoviruses, a family of non-enveloped DNA viruses occurring in vertebrates, including humans. Adenoviruses have been the focus of intense research for more than 67 years. Besides causing disease, they have immensely contributed to the advance of life sciences and medicine over the past decades. Recently, adenoviruses have been widely used as vehicles in gene therapy and vaccination. They continue to provide fundamental insights into virus-host interactions in cells, tissues and organisms, as well as systems and metabolic networks. This special issue of FEBS Letters presents a unique collection of 23 state-of-the-art review articles by leading adenovirologists. In this prelude, I present the chapters, which provide a solid basis for further exploring the rich heritage in adenovirus molecular cell biology, structural biology, genetics, immunology, gene therapy and epidemiology. I conclude with an essential discussion of six blind spots in adenovirology.

Introduction

Adenoviruses (AdVs) are non-enveloped, double-stranded DNA viruses of vertebrates. They are abundant in fish, amphibia, reptilia, birds and mammals, including humans. Currently, about 110 human adenoviruses (HAdV) types and genotypes are known and classified into seven species (A-G). AdVs infect the respiratory organs, the eyes, the kidney, the gastrointestinal tract and blood cells. On a population scale, AdVs emerge unpredictable and can cause human epidemics. The structure of the virion is related to phages of eubacteria and archea, and is part of an evolutionary lineage comprising the internal membrane-bearing PRD1 phage of the *tectiviridae* infecting gram-negative bacteria, paramecium bursaria chlorella virus (PBCV) infecting green algae, sulfolobus-turreted-icosahedral-virus (STIV) infecting archea, as well as vaccinia virus, an attenuated form of the cowpox disease virus, related to variola virus causing small pox [1-5]. Both AdVs and PRD1 have a similar icosahedral capsid organisation, and a structurally related major capsid protein, as indicated by cryo-electron microscopy and X-ray diffraction studies [6, 7].

At first glance it appeared that the structure of AdV bears no more secrets, but a closer look reveals some surprises. For example, the observation that a HAdV-C5 mutant lacking the major DNA-associated viral protein VII failed to escape from endosomes during entry was counterintuitive [8]. Yet, it provided unexpected insights into intra-virion competition between the VII and the membrane lytic protein VI, which is necessary to expose enough VI from the incoming virion, such that the endosomal membrane ruptures and infection can occur [8-10]. Both PRD1 and AdV have a double-stranded DNA genome with inverted terminal repeats, and a terminal protein at both 5’ ends, but the genomes have no similarities otherwise. The genome length of AdVs ranges from 28 to 48 kbp, and it appears to be adjusted to match the size of the virion capsid [reviewed in 2, 11, 12].

Cell entry

In this Special Issue of FEBS Letters, Glen Nemerow and Jane Flint describe in detail the structure of the HAdV particle, and give a historical account on the fascinating highlights in AdV research throughout the past 50 years [13]. The best-studied HAdV genetically and biologically are the species C types 2 and 5. They use the Coxsackie and AdV receptor (CAR) as an essential receptor for entry [14]. CAR mediates cell-cell contacts and is essential for immune-cell activation, synaptic transmission, and signaling [15-18].

The review by Kate Excoffon highlights the many roles of CAR in AdV and coxsackie virus infections and in development, and describes how alternatively spliced mRNAs encode CAR variants that are trafficked differentially to the basolateral or apical surface of polarized human epithelial airway cells [19]. This question of trafficking is important, since CAR localizes to the basolateral surface of polarized cells, and environmental viruses access the airways from the apical side. Interestingly, CAR can also be trafficked to the apical plasma membrane upon release of chemokines triggering G-protein coupled receptor signalling by activated macrophages, and this then provides entry gates for apical entry of HAdV-C [20, 21]. Downstream cell entry steps of HAdV-C are well studied [22]. Entry requires host cues dismantling the incoming particle in a stepwise manner [23]: the particle escapes from the endosomal compartment, yet remains stable enough to traffic the cytosol and deliver the viral genome to the nucleus [22, 24, 25].

Transport of virus particles to the nucleus occurs on intact microtubules by the minus end-directed dynein-dynactin motor complex, antagonized by the plus end-directed kinesin motor [26-29]. Bidirectional transport is regulated by MAPK and protein kinase A (PKA) signalling downstream of integrin activation by incoming virions [30, 31]. While PKA activation drives HAdV-C to the center of non-polarized cells, it disperses endo-lysosomes to their periphery [32]. Scherer and colleagues now explain how HAdV-C interacts with the dynein motor complex. They discuss how virus-dynein interactions could be regulated by phosphorylation, in particular, how PKA releases dynein from Rab7-interacting lysosomal protein (RILP) on late endosomes/lysosomes and makes phosphorylated dynein motor available for binding to the incoming AdV particle [33]. It remains to be explored if transport of RILP-positive vesicles to the cell periphery leads to lysosomal fusion with the plasma membrane and the release of acid sphingomyelinase, which is known to occur early in HAdV-C entry [34]. Alternatively, RILP-containing vesicles support autophagic clearance of cytoplasmic entities, and they might be part of a sterilizing mechanism clearing HAdV particles from the cytosol [35]. The review also discusses the roles of kinesin-1 motors in HAdV-C entry, Kif5B transporting the virions to the cell periphery, and Kif5C dismantling the particles tethered at the nuclear pore complex for releasing the viral DNA into the nucleus [25, 36].

Imaging and ‘Omics

Historically, initial insights into cell and virus trafficking were derived from fluorescence imaging of single particles, and HAdVs were at the forefront in this field [37, 38] (reviewed in [39, 40]). Noemi Pied and Harald Wodrich describe how advanced fluorescence microscopy revealed virion capsid proteins, the viral DNA and also non-

structural regulatory viral proteins involved in replication and immune defence [41]. It is discussed how different imaging modalities and tracking algorithms are used to analyse viral surfing on the plasma membrane, endocytosis, endosomal escape, transport in the cytoplasm and DNA nuclear import [42-47]. The authors discuss the question of viral DNA remodelling in the nucleus based on live and static imaging, viral transcription visualized by fluorescence *in situ* hybridization and the establishment of replication compartments, where viral DNA and proteins give rise to progeny particles [48]. The impact of anti-viral immunity in the nucleus, including the DNA-damage response is discussed, and advanced technology, including correlative light and electron microscopy, light-sheet microscopy and machine learning are introduced to highlight the power of microscopy in single-cell analyses of HAdV infections.

Zhao and colleagues enrich the topic of cell biology and molecular imaging with large-scale 'omics technologies and focus on the transcriptional landscape in the infected cell, which is at the heart of reprogramming the host-cell proteome during lytic course of infection [49]. Notably, AdV research has been at the forefront of the field by providing an integrative view of transcriptomics with proteomics, and showcased how quantitative RNA profiling and proteome-wide analyses give insight into protein turnover [50]. An interesting notion that derives from such combinatorial analyses is that host proteins regulating immune response, such as NFκB, STAT or p53, are upregulated early in infection, but their downstream effectors are downregulated later in the infection. One explanation is that AdV early proteins, such as the immediate early transactivator E1A, inhibit these responses, for example by sequestering the p300/CBP transcriptional coactivators [51, 52] or by interaction with the hBre1/RNF20 complex blocking interferon (IFN), and induce histone 2B ubiquitination along with suppression of IFN-stimulated gene (ISG) expression [53]. This downregulation is enhanced by E1B-55K, which inhibits ISG expression and STAT signalling [54].

Extracellular restrictions

Viruses not only face intracellular but also extracelluar restriction. The comprehensive review by Rondine Allen and Andrew Byrnes provides a fascinating picture of the many-fold interactions that AdVs engage with antibodies, complement and coagulation factors [55]. The authors explain how natural preexisting IgM and virus specific IgGs neutralize virus particles outside of cells before activation of T cells. Remarkably, antibodies also have direct intracellular anti-viral effects. They are piggybacked on virions and delivered in complex with virions to the cytosol, where they provide signals for the recruitment of the TRIM-21 ubiquitin ligase, the p97/VCP ATPase and the proteasome, which inactivate

the virus [56]. Anti-AdV IgGs, the preexisting ones included, account for inhibitory effects against AdV-based vaccines. The review also describes in detail how the complement system inhibits AdV infection, and that the coagulation factor X shields HAdV-C5 from attack by natural antibodies and complement [57].

Numerous insights were further derived from studies of HAdV in mice. Systematic work investigating the interactions of HAdV with blood factors has revealed significant therapeutic implications not only for AdVs but virtually any other virus or exogenous vector in clinical settings. Investigations of the HAdV interactions with mice have immensely contributed to our understanding of viruses and innate immunity. The review by Svetlana Atasheva, Jia Yao and Dmitry Shayakhmetov describes the plethora of systemic responses occurring in mice inoculated with HAdV [58]. The activation of the innate immune system leads to a cytokine storm syndrome, disseminated intravascular coagulation, thrombocytopenia, and hepatotoxicity, and may cause morbidity and mortality. Detailed studies of the underlying mechanisms have taken advantage of virus and host genetics, and greatly improved our understanding of mechanisms underlying activation of innate immunity against HAdV.

Intracellular restrictions and viral countermeasures

To overcome the innate restrictions imposed by host defence, AdVs express a range of multifunctional proteins and RNAs to maintain the viral genome in the infected cell despite anti-viral cytokine responses. At reduced restriction, this promotes the synthesis of viral progeny. Sook-Young Sohn and Patrick Hearing show how HAdV antagonizes the innate cellular reactions, including IFN response by suppressing the ISGs like PML, and DNA damage response (DDR) by inhibiting DDR sensors, such as the Mre11-Rad50-Nbs1 complex, and various DDR effectors [59]. In many cases, these HAdV reactions to innate immunity are common with many other viruses, and provide a blueprint to decipher general virus-host interactions.

An important role in the viral immuno-modulation is attributed to the E3 region of the HAdV genome. This region contains a handful of open reading frames (ORFs) that diverge in different HAdV species and are evolutionarily adapted to the host immune defence of the natural hosts and their tissues. Edson Oliveira and Marlene Bouvier discuss the subversion of host antiviral immune responses by HAdVs, and highlight the suppression of MHC class I antigen presentation in the infected cells [60]. Among the better understood ORFs is the one encoding the E3-19K glycoprotein, which binds to human leukocyte antigen (HLA) 2 in the endoplasmic reticulum (ER) and prevents

surface transport of HLA and recognition of the infected cells by cytotoxic T cells [61, 62]. E3-19K also activates the unfolded protein response (UPR) sensor in the ER and contributes to lytic and persistent HAdV-C infection [63].

Fanny Georgi and Urs Greber review how the E3 AdV death protein (ADP) controls lytic infection [64]. ADP is specific for HAdV-C, is comprised of about 100 amino acids, harbours a single membrane-spanning segment. ADP undergoes post-translational processing in ER and Golgi compartments, before it localizes to the inner nuclear membrane, where it induces membrane rupture at late stages of infection [65]. ADP provides also an interesting option to control cell death of cancer cells, but how it induces cell lysis is incompletely understood.

Nuclear events and transformation

Besides the immediate early AdV transactivator E1A, another early protein, E1B-55K plays an important role in suppressing innate immunity, and additionally contributes to cell transformation. Wing Hang Ip and Thomas Dobner discuss the evidence for how the proteins encoded in the E1 and E4 regions of the virus immortalize primary cells [66]. Exciting new findings from the laboratory of Arnie Berk recently revealed yet another surprising connection between E1A and cell transformation, namely that E1A dedifferentiates cells by the inactivation of the Hippo pathway effectors YAP and TAZ [67]. The silencing of E1A in human embryonic kidney 293 cells dramatically changed their morphology and gave rise to a gene expression profile akin to mesenchymal stem cells. The data show that E1A modulates a developmental checkpoint controlled by YAP/TAZ, prevents differentiation of progenitor cells and thereby renders the cells conducive for virus production.

Paloma Hidalgo, Wing Hang Ip, Thomas Dobner and Ramon Gonzalez discuss how posttranslational modifications, such as SUMOylation and phosphorylation, regulate E1B effector protein binding in a spatio-temporal manner, throughout different phases of infection [68]. This involves the establishment of viral replication compartments, viral genome replication, transcription, degradation of cellular proteins, and viral late mRNA biogenesis in the nucleus.

The biochemical and cell biological processes in the formation of the AdV replication compartments in the nucleus are described by Paloma Hidalgo and Ramon Gonzalez [69], and by Matthew Charman, Christin Herrmann and Matthew Weitzman [70]. These two reviews summarize recent findings describing the complexity of viral DNA replication in the midst of host chromatin dynamics. Notably, the replication compartments are the

sites for viral gene expression. They are positioned near the virion assembly sites, where viral capsomers, scaffold proteins, and viral DNA protein complexes coassemble to give rise to viral particles [71]. Remarkably, the protein VII knock-out mutant HAdV-C5-dVII is devoid of the major viral DNA-binding protein, yet, it forms virions indistinguishable from wild type [8]. This argues that HAdV-C5 forms particles by a coassembly process rather than drilling the viral DNA-protein complex through a portal structure, as observed with bacteriophages or herpes viruses.

The review by Kelsey Lynch, Linda Gooding, Charlie Garnett-Benson, David Ornelles and Daphne Avgousti sheds light on how HAdV replication, and how this is coupled to epigenetic chromatin modulation, in the context of E1A and protein VII [72]. They also discuss how E4orf3 helps to exclude host chromatin from sites of viral replication.

The contribution by Tamar Kleinberger discusses the function of the viral E4orf4 protein in infection and cell transformation [73]. E4Orf4 is a small 14 kDa protein, which periodically cycles throughout the infection. It controls infection by downregulating early viral gene expression and altering the splicing patterns of viral mRNAs in a time-controlled manner. E4Orf4 activates mTOR, enhances viral protein production, and inhibits the host DNA damage response, an antiviral defence mechanism. E4Orf4 also binds to numerous cellular proteins, including protein phosphatase 2A (PP2A). When expressed alone, it induces cancer-selective cell death, which makes it an interesting candidate in cancer therapy.

Persistent and chronic infections

HAdVs are recognized as severe pathogens in immunosuppressed individuals [74]. They emerge unpredictably, as indicated by recent outbreaks of HAdV-B3 and -B7 in New Jersey, USA [75]. The currently available medications against HAdV based on nucleoside analogues are unfortunately largely ineffective. Epigenetic silencing of HAdV genomes may contribute to viral persistence in lymphoid cells. Thomas Lion explains how HAdV persistence gives rise to clinical pathology in immuno-suppressed people [76]. Acute infections of the ocular mucosa occur predominantly with HAdV-D, and lead to clinical eye pathogenesis. Ashrafali Mohamed Ismail, Xiaohong Zhou, David Dyer, Donald Seto, Jaya Rajaiya and James Chodosh describe the clinical pathology of the disease and delineate the molecular phenotypes of the viruses causing epidemic keratoconjunctivitis (EKC), a highly contagious ocular surface infection, which can lead to chronic, recurrent or visually disabling keratitis [77].

Vectors and therapy

Michael Barry, Jeffrey Rubin and Shao-Chia Lu provide an impressive account of the therapeutic potential and the pharmacology of HAdVs. They show how the diversity of AdVs, the AdV virome, is being harnessed to develop suitable vectors for specific applications [78]. The authors emphasize that successful use of therapeutic viruses involves not just accurate targeting of the vector to the tissue of interest, but also requires sufficient vector detargeting, for example by shielding nonessential domains of the virion from attack by complement and uptake by liver macrophages [79].

To take full advantage of the power of AdV vectors in targeting and delivery to cells and tissues, efficient and accurate cloning and engineering technology must be in place. Jian Gao, Kemal Mese, Oskar Bunz and Anja Ehrhardt provide a comprehensive update on HAdV vectorology, and highlight some of the therapeutic options that can be envisioned with these procedures [80]. The list of currently available vectorized HAdVs and translational medical applications of these vectors is impressive.

One of the most promising applications of HAdV vectors lacking any viral sequences except the inverted terminal repeats is the transduction of hematopoietic stem cells (HSCs) for genome editing for a range of haematological genetic diseases. HSCs can self-renew and differentiate into all blood cell lineages. Chang Li and André Lieber present the state-of-the-art genome editing of HSCs with helper-dependent gutless HAdV delivery vectors [81]. Applications in curing inherited disorders now become possible, for example in haemoglobinopathies, and a range of infectious diseases, including AIDS, caused by human immunodeficiency virus. The authors review the pros and cons of expression of designer nucleases and base editors from episomal DNA, transposase-mediated random integration, and targeted repair by homology-dependent integration into genomic loci of choice.

Animal AdVs

The Special Issue closes with two chapters on animal AdVs. They highlight the similarities and remarkable differences between human and animal AdVs, their evolution, and the potential threats to humans from zoonotic transmissions. Silvio Hemmi and Katherine Spindler review the current essential knowledge of murine adenoviruses, including mouse adenovirus type 1 (MAdV-1), but also MAdV-2 and MAdV-3, the last two belonging to the murine mastadenovirus species B and C, respectively [82]. The review focusses on MAdV-1, a member of the murine mastadenovirus species A, and describes the molecular genetics, virus life cycle, cell and tissue tropism. They also describe host

immune responses to MAdV-1, viral persistence, and host genetics of susceptibility. The authors show how studies of these viruses inform about pathogenesis in a natural host. They close the loop by reporting how MAdV vectors and vaccines are being developed for studies in murine therapy and disease models. Together, the topic is relevant because small animal models have limitations for studies of pathogenesis caused by HAdVs, perhaps with an exception of Syrian hamsters, which allow the replication of HAdV-C and certain B species types [83, 84].

AdVs occur in many different vertebrate species, albeit at variable extent. Balazs Harrach, Zoltan Tarjan and Maria Benkö provide an up-to-date overview of the known AdVs in birds, reptiles, and bats which host the most common and diverse AdVs, while only a few AdV types are known from fish and amphibians [85]. The authors also discuss the mastadenoviruses and atadenoviruses in mammals, and describe the phylogenetic relationships of the different types. Interestingly, they explore the concept that AdVs have co-evolved with their respective hosts, and are adapted to the corresponding physiology and immune systems. They put up a most likely scenario for AdV evolution, which implies a long-term co-speciation with the respective hosts, as well as occasional switches between closely or, rarely, more distantly related hosts. This has implications for cross species AdV transmissions, including zoonoses.

Conclusions – Six blind spots in adenovirology with impact beyond molecular cell biology

As illustrated above, AdV research has been a major driver of molecular cell biology and provided insight into disease mechanisms. This series of review articles emphasizes the necessity to better understand the nature of host genetic and biochemical variability at the cellular and the organismic levels. The challenge now is to tie this knowledge into concepts, and develop new biology and therapies, both with and against the virus. I can think of six major areas that are attractive for future investment.

One: Metabolism

An area with big open questions is how the virus interlinks with host cell metabolism [see for a recent review 86]. For instance, E4Orf1/6 activates c-Myc and phosphofructokinase / hexokinase and thereby boosts glycolysis and glutaminolysis [87]. This loop may further connect to the nutrient sensing mTOR pathway, which affects nucleotide metabolism and thereby positively influences viral DNA replication in the nucleus [88, 89]. These are important hallmarks not just for viral disease, but also cancer progression [90, 91]. In fact, Otto Warburg observed early on that cancer cells converted the majority of glucose to

lactate, despite the presence of oxygen [92]. It may also be interesting to deepen investigations of lipid metabolism affecting viral propagation and dissemination [34, 93]. Of interest may further be to explore how metabolism controls the transcriptional feedforward loop involving the E1A, E3B-19K, IRE1α, XBP1s, as well as the XBP1s binding sites on the E1A enhancer / promoter in HAdV-C persistence and lytic outbreak [63]. Additionally, it will be important to study transcript regulation, for example by microRNAs regulating host and viral transcripts, and complex RNA networks including long noncoding RNAs [94].

Two: Virus egress

A significant blind spot in adenovirology is virus egress from the infected cell, and if this involves lytic and / or non-lytic pathways. Lytic pathways have been attributed to the AdV death protein, a small type III transmembrane protein that exclusively occurs in the AdV-C types [2, 64, 65, 95]. It has remained unknown, however, if other AdVs spread by lytic or by non-lytic egress. The former would be pro-inflammatory and the latter anti-inflammatory. Procedures based on machine-learning of infection features can prospectively identify lytic and non-lytic AdV-infected cells [96]. They provide a handle to delineate not only the phenotypes but also the mechanisms of the lytic and potentially non-lytic egress pathways. Emerging knowledge in this field will be important for disease management and also to improve AdV-based oncolytic therapy.

Three: Protective host factors and infection variability

A key challenge for future research will be to shed light on the nature of novel protective networks in cells against AdV infection, and to sort out their strengths and weaknesses at the cellular and organismic levels. This will involve investigations of the variability of the inflammatory nature of AdVs. For example, computational genomics data indicate that past, present and future viruses are major drivers of host evolution, and that at least 30% of the adaptive amino acid changes in the human proteome conserved in mammals are due to viruses [97]. Developing tools and assays to explore infection variability will provide a fertile ground for future discoveries in host-pathogen interactions.

Four: Epidemiology and zoonoses

In direct relation to point three, and not surprisingly, a recent systematic survey of the epidemiologic and zoonotic potential of AdVs has shown that AdVs have crossed species barriers to humans multiple times. Especially non-human primates but also other animal species such as bat, feline, swine, canine, and ovine crossed species barriers, and this

likely will occur again in the future [12]. This is an indication that we not only need pan-species diagnostics to detect new recombinant AdVs, but also increase the awareness of clinicians about the latest technologies to identify potential threats to humans before the threats become reality.

Five: New AdVs

Novel human and animal AdVs will need to be further identified and characterized not only bioinformatically and with 'Omics technology, but also with regards to their potential for zoonotic and anthroponotic transmission across the species barriers. We need to better understand the major species barriers, in particular the innate immune system. It is likely that an extensive yet-to-be-discovered network of proteins and nucleic acids protects our cells from attacks by AdVs, beyond to the already known anti-viral factors. It is crucial to understand that when viruses change their host, they break through this network and cause disease in unprecedented ways, often also by acquiring new functions. In fact, molecular evolution studies of HAdV-C have pointed to frequent recombinations in viral genomes as likely drivers of pathogenesis [98].

Six: AdVs in therapy

A challenge with practical implication is to enhance the safe use of human and non-human AdVs in therapy. This will require a broad mapping of pre-existing humoral immunity against AdVs [99]. This is especially important if AdV-based vaccines are to be introduced into the human population. Attempts with chimpanzee-derived AdV vectors against MERS coronaviruses are promising [100, 101], and phase I/II clinical trials with ChAdOx1-nCoV-19 against SARS-CoV-2 have commenced in the UK, as based on virus-reducing and pneumonia-preventing effects in vaccinated rhesus macaques [102, 103]. All these challenges will require continued support for basic research in adenovirology, including immune cell interactions, the effect of preexisting T cells, and detailed studies of rare HAdV types.

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